Rare alleles and level of inbreeding in five chicken populations reared in Ogun and Ondo states of Nigeria as revealed by microsatellite markers

Oni O.A[1,2*], Olowofeso O[1], De Campos J.S[1] and Emiloju C.O[3]

[1]Department of Animal Breeding and Genetics, Federal University of Agriculture, P. M. B. 2240, Abeokuta, Nigeria.

[2]Agricultural and Natural Resource Department, Ondo State Local Government Service Commission, Akure, Nigeria.

[3]Department of Biological Sciences, Covenant University, Otta, Ogun State, Nigeria.

Abstract- Microsatellite markers MCW88, MCW150, ADL171 and ADL206 were used to analyze genetic variability and divergence of chicken populations of different geographical location in Ogun and Ondo States, Nigeria. The chicken populations included 16 Frizzle feathered (Ff), 16 Normal feathered (Nf), 16 Naked neck (Nn), 16 Bovan nera (Bn) and 16 Black harco (Bh) making a total of 80 isolated genomic DNAs. Sample of DNAs were amplified by polymerase chain reaction (PCR) and separated on 1% agarose gel. 84 alleles were detected by the selected markers. The number of identified alleles per locus ranged from 2 to 14. High value of F_{ST} (0.1167) across loci revealed a substantial degree of population differentiation. In the majority of the chicken populations high heterozygosity and some level of inbreeding were determined. The heterozygosity ranged from 0.5315±0.08 to 0.6989±0.03 while inbreeding ranged from 0.2168±0.19 to 0.4448±0.09. The Nei's chord genetic distance (D_a) was also calculated using Microsatellite Analyzer. Dendrogram developed using neighbor-joining method showed that Bovan nera(Bn) vs. Black harco(Bh) formed the first group (0.1404), followed by Frizzle feathered (Ff) vs. Bovan nera(Bn) (0.1825), Frizzle feathered(Ff) vs. Normal feathered(Nf) (0.2255), Normal feathered(Nf) vs. Naked neck(Nn) (0.3699), respectively. These results highlight the applicability of microsatellite markers to determine rare allele, inbreeding coefficient and genetic divergence in five chicken populations in Ogun and Ondo States, Nigeria. It was concluded that the markers were useful in revealing genetic information present in the five chicken populations.

Keywords: Chicken populations, genetic divergence, microsatellite, phylogenetic relationship

I. INTRODCTION

An allele is one of a number of alternative forms of the same gene or some genetic locus. Sometimes, different alleles can result in different observable phenotypic traits, such as different pigmentation. However, most genetic variations result in little or no observable variation. Traditionally rare alleles have been defined in term of their frequency. Paul Joyce and Simon Tavares (1995) defines rare variant as an allele with relative frequency of less than q (average frequency), for some small pre-specified value of q such as 0.01. The inbreeding coefficient of an individual is the probability that two alleles at any autosomal locus are identity by descent (ibd). Two alleles are ibd if one is physical copy of the other or if they are both physical copies of the same ancestral allele. The inbreeding coefficient is often calculated from pedigree information, but it is also possible to estimate inbreeding using information from genetic markers. In the absence of pedigree information, genetic markers have been used extensively to estimate relatedness between individual and the level of inbreeding in population (Olowofeso et al., 2016; Al-Qamashoui et al., 2014; Ardeshir et al., 2012). Many genetic diversity variables and approaches can be achieved by using microsatellite markers. These include allele frequencies, inbreeding coefficient, rare alleles, proportion of polymorphic loci, observed and expected heterozygosity, phylogenetic relationship, genetic admixtures and structure (Olowofeso et al., 2016; Miao et al., 2013; Leroy et al., 2012; Getachew et al., 2016). Microsatellite have become the preferred type of genetic markers because of their abundance, random distribution, co-dominant inheritance, high variability, possibility of automated detection Goldstein and Pollock (1997) and ability to determine heterozygosity and genetic distance based on microsatellite analyses is regarded as the most convenient method. Report show that microsatellites are excellent genetic markers because of their locus identity, high PIC value, widespread distribution in the genome, multi-allelisms and their co-dominant nature (Olowofeso et al., 2005; Warren et al., 2008; Ohwojakpor et al., 2012). The high variability of microsatellite and their distribution give them advantages over other markers. The genetic parameters of chicken populations were examine using decamer and microsatellite markers by Oni et al. (2016). Microsatellites have been effective in evaluating genetic differences between and within breed of chicken and also to determine population sub structure (Adeleke et al., 2011; Olowofeso et al., 2011).The objective of the present study was to determine the applicability of microsatellite markers with chicken populations to know the level of inbreeding, rare alleles and genetic divergence of four microsatellite markers used with the five chicken populations in Ogun and Ondo States, Nigeria.

II. MATERIALS AND METHODS

Blood collection, location and sample size

Blood samples were collected from different geographical location in Ogun and Ondo States, Nigeria using random sampling by brachial venipuncture aseptically into a labelled haemotocrit tube containing ethylene diamine tetra acetic acid (EDTA) used as an anticoagulant. Approximately 2ml of blood was collected from each of the birds using 5ml disposable syringe, the blood was

transferred to the laboratory for DNA extraction. Analyses were carried out at the Biotechnology centre, Federal University of Agriculture, Abeokuta, Nigeria. The sample size by population were Frizzle feathered (16), Normal feathered (16), Naked neck (16), Bovan nera (16), Black harco (16), respectively, making a total of 80 chicken populations.

DNA isolation, PCR protocol and electrophoresis

Individual DNA was isolated from chicken blood collected using bench protocol, DNA quality was checked by electrophoresis in a mini gel and quantified using a spectrophotometer based on absorbance at 260nm and 280 nm respectively, each DNA was adjusted to 100 ng/µL. Genotyping of DNA samples at the 4 microsatellite markers were carried out using the isolated DNA from five chicken populations. The sequences of the microsatellite markers were replica of those used by Oni et al. (2016) and coded MCW 150, MCW 88, ADL 206, ADL 171. The microsatellite (MS) PCR component in each micro-PCR tube, contains 1 µL template DNA, 2.5 µL of 10 x PCR buffer, 1 µL of 25 mm dNTP, 2 µL (1 µL forward and 1 µL reverse form) of each pair of primers, 0.2 µL of (5 U/µL) Taq DNA polymerase (enzyme), 2.2 µL of 25 mm/molMg2+ with 16.1 µL sterilised distilled water added. The reaction programme was as follow: initial denaturation (940C, 300 s), 35 cycles of denaturation at (940C, 60 s), annealing temperature ranged between 460C and 550C for 60 s and extension at (720C, 60 s), followed by final extension at (720C, 600 s). The fragments amplified by PCR were separated by gel electrophoresis using 1% agarose gel with a drop of (0.5 µL) ethidium bromide used as staining agent. Exactly 1 µL of mobility marker was place on cellophane paper and 10 µL of amplified products were added, mixed and loaded into capillary well of prepared gel, the buffer used in the gel was 1 x TBE, while 10 µL of PBR322DNA/Msp1 was used as ladder to estimate the size of the amplicons. The electrophoresis lasted for 1 hour at 100 V and 20 mA.

Statistical Analysis

The microsatellite data obtained with the five chicken populations were analyzed with Microsatellite Analyzer (MSA) Version 4.05 developed by Dieringer and Schlotterer (2003) to obtained allele frequency, allelic richness, unique allele, F Statistic and inbreeding coefficient. The unbiased genetic distances (DN) were also obtained with the use of Microsatellite Analyzer. The results were used to construct the dendrogram by neighbour-joining systematic analysis.

III. RESULTS AND DISCUSSION

Allele size and frequency distribution

Figures 1 to 4 represented the allele sizes and frequency distribution at the four microsatellite markers used with the five Nigerian chicken populations. 84 alleles were detected by the selected markers. The number of identified alleles per locus ranged from 2 to 14. Six alleles were produced by marker MCW 150 and the allele size ranged from 145 to 155 base pairs (Figure 1). The highest frequency of 0.6667 at 147bp band was observed in Normal feathered chicken population, while Bovan Nera chicken population recorded the lowest of 0.0333 at 145bp band. The bands frequency of 147bp, 149bp and 151bp fragments were similar across Naked neck, Bovan nera and Black harco chicken population. 147bp and 151bp allele were present in all the chicken populations (Frizzle feathered, Normal feathered, Naked neck, Bovan nera and Black harco). The band size of 145bp, 149bp and 153bp were found in Bovan nera. No band was recorded for Frizzle feathered, Normal feathered, Naked neck and Black harco at 145bp, 149bp and 153bp; Frizzle feathered and Naked neck at 155bp. Allele frequencies data for microsatellite MCW 88 were shown in Figure 2. Five alleles were detected by MCW 88 which ranged from 196bp to 204bp. The highest frequency of 0.7857 at 200 bp was observed in Naked neck, while Frizzle feathered and Bovan nera recorded the lowest frequency of 0.0313 at 200 and 202 bp. No band was detected for Normal feathered at band 196bp and 204bp; Naked neck at 196bp and 198bp; Frizzle feathered at 198bp, 202bp and 204bp; Bovan nera and Black harco at 204bp. The bands of 200bp were detected in all the populations. Allele frequency data for the microsatellite ADL 206 were shown in Figure 3. ADL 206 amplified 5 alleles which ranged from 184bp to 204bp. The Frizzle feathered recorded the highest frequency of 0.8077at 184bp, while the lowest frequency of 0.0416 was recorded at 200bp by Normal feathered chicken population. The bands of 184bp and 186bp were detected in Frizzle feathered; 184bp, 200bp and 202bp were detected in Normal feathered; 202bp and 204bp were detected in Naked neck; 184bp, 202bp and 204bp were detected in Bovan nera; while 184bp, 200bp, 200bp and 204bp were detected in Black harco. No band was detected for Frizzle feathered at 200bp, 202bp and 204bp; Normal feathered at 186bp and 204bp; Naked neck at 184bp, 186bp and 200bp; Bovan nera at 186bp and 200bp; Black harco at 186bp. The allele frequencies determined for microsatellite ADL 171 are shown in Figure 4. For ADL 171, there were 14 alleles which range from 180bp to 204bp. Allele frequency was greatest at 0.5385 for Naked neck chicken population at 190bp. The lowest frequency of 0.03125 was observed in Bovan nera at 202bp and 204bp. No band was observed at 180bp, 182bp, 184bp, 186bp, 188bp, 189bp, 190bp and 192bp for Frizzle feathered; 189bp, 190bp, 192bp and 202bp for Normal feathered; 180bp, 182bp, 184bp, 194bp, 196bp, 198bp, 200bp, 202bp and 204bp for Naked neck; 180bp, 182bp, 184bp, 186bp, 188bp, 189bp, 190bp and 194bp for Bovan nera; 180bp, 182bp, 184bp, 186bp, 188bp, 189bp, 190bp, 192bp and 194bp for Black harco.

Rare/Unique alleles

Tables1-4 shows the observed alleles unique to certain populations. A total of 10 unique alleles were observed. For Bovan nera, a total of 2 unique alleles were observed in MCW 150 (Table 1). Table 2 shows 2 unique alleles in MCW 88, 1 unique alleles each was observed in Normal feathered and in Naked neck. In ADL 206, only 1 unique allele was observed in Frizzle feathered (Table 3). The Normal feathered had 3 unique alleles in ADL 171, while Naked neck had 2 unique alleles also in ADL 171 (Table 4). Consequently, unique alleles could be of importance in the improvement of future strategies towards genetic typing and line identification most especially if occur in relatively high frequency as earlier reported by Toro *et al.* (2006). It was also pointed out by Wimmers *et al.* (2000) that unique alleles could be utilized as population fingerprint even at one allele per locus. The total number of unique alleles in this study is lower than the numbers (13) reported by Roushdy *et al.* (2008) for Egyptian Fayoumi

native breed with six microsatellite markers. Such differences could be attributed to differences in sample size as well as characteristics of microsatellite markers employed. Hillel *et al.* (2003) reported a proportion of rare alleles greater than 10%. Olowofeso *et al.* (2016) also detected 20.29% rare alleles in Nigerian chicken populations confirming the suitability of microsatellite markers in detecting rare alleles in chicken populations. In this studied, 33.33% rare alleles were detected indicating that the four microsatellite markers were suitable for detecting rare alleles in chicken populations, highest number of rare alleles was observed in Normal feathered chicken and can be selected for increased production ahead of other chicken populations examined.

F Statistics

Table 6 reveals the values of global F_{ST}, F_{IT}, F_{IS}, G_{ST} and N_m over all loci considered in this study. The mean value of correlation of genes of different individuals in the same population (F_{ST}) was 0.1667±0.04; the correlation of genes within individual over the all population (F_{IT}) had a mean value of 0.4549±0.09, while correlation of genes within individuals within population (F_{IS}) had a mean value of 0.3509±0.10. The mean value of F_{ST} (0.1677) across loci revealed a substantial degree of population differentiation. Genetic differentiation (G_{ST}) and gene flow rate/migration rate (N_m) had a mean value of 0.1778±0.03 and 1.2632±0.44, respectively. The highest value of 0.2284 was observed in F_{ST} at locus MCW 88, while the lowest value of 0.0880 was detected in locus MCW 150. The highest value of 0.6994 was detected in F_{IT} at locus ADL 206, while the lowest value of 0.2572 was seen in locus ADL 171. Locus ADL 206 had the highest value of 0.6117 at F_{IS} while locus ADL 171 had the lowest value of 0.1514 at F_{IS}. G_{ST} values recorded ranged from 0.1075 at locus MCW 150 to 0.2424 at locus ADL 206 and N_m values ranged from 0.8446 in locus MCW 88 to 2.5909 in locus MCW 150, respectively. This studied shows that migration rate (N_m) depends mainly on F values and a lower value of F brings about higher value of N_m or vice versa. Gene flow occurred among chicken populations because the calculated migrant rate for each marker across population was more than zero. High value of fixation indices (F_{IT} and F_{IS}) observed indicates that inbreeding occurs within these chicken populations.

Inbreeding coefficient (F_{is})

Table 7 shows the value of inbreeding coefficient (F_{is}) using four microsatellite loci. The highest mean value of potential inbreeding of 0.4448±0.09 was observed in Frizzle feathered population, while the lowest mean value of 0.2168±0.19 was observed in Black harco population. The F_{is} value detected in the Normal feathered, Naked neck and Bovan nera chicken populations were 0.4105±0.22, 0.2954±0.04, 0.3406±0.18, respectively. The inbreeding coefficient (F_{is}) which suggested the potential reduction in heterozygosity due to non-random mating and may serve as an indication of inbreeding within the population. In the present study, the F_{is} value was detected in all the chicken populations. This indicated that inbreeding occur the population sampled, resulting in more related bird than expected. The inbreeding could be partly due to the fact that the chicken populations in this study have been subjected to some form of selection over time or partly due to maternal effect.

Heterozygosity (H_S)

Table 9 shows the gene diversity in the five chicken populations which reflected the heredity and mutation of loci in each group. The highest value of 0.6989±0.03 was observed in Bovan nera. This was followed by Black harco, Normal feathered, Frizzled feathered and Naked neck with values of 0.6976±0.02, 0.6147±0.09, 0.5450±0.10 and 0.5315±0.08, respectively. The mean values across populations ranged from 0.5497±0.08 in ADL 206 to 0.7543±0.04 in ADL 171. The highest value of 0.8730 was observed in Normal feathered in locus ADL 171, while the lowest value of 0.3185 was observed in Frizzle feathered in locus ADL 206. Considering the number of allele, markers, high heterozygosity and level of inbreeding within the chicken populations it means that the markers were useful in revealing genetic information present in these chicken populations.

Genetic distance and phylogenetic relationship

The Nei's chord genetic distance was presented in Table 8. The smallest genetic distance was obtained between Bovan nera vs. Black harco (0.1404) while the largest genetic distance was found between Normal feathered vs. Naked neck (0.3699). Bovan nera vs. Frizzle feathered and Frizzle feathered vs. Normal feathered had genetic distance of 0.1825 and 0.2255, respectively. Using these distances obtained, the dendrogram (Figure 5) was constructed using the neighbour-joining method. In the dendrogram, Bovan nera and Black harco formed the first clustered group with the smallest genetic distance and have the smallest measure of relatedness, followed by Frizzle feathered and Normal feathered and Naked neck respectively. Naked neck was the most distantly related among the examined chicken.

IV. CONCLUSION

The results of this research confirm the usefulness of microsatellite markers for the research of genetic variation and divergence in five chicken populations. Although, only a small number of microsatellite markers were used, relatively reliable results were obtained that is markers were useful in revealing genetic information present in the five chicken populations. This study revealed the level of inbreeding, proportion of rare alleles (33.33%) and common alleles (66.67%) present in the chicken. The inbreeding coefficient in the chicken ranged from -0.0276 in Bovan nera population detected by ADL 171 to 0.8511 in Normal feathered population revealed by ADL 206, some of the markers produce negative value within the chicken populations but the fixation index (F_{IS}) of other markers were greater than zero, an indication that inbreeding occur in the five chicken populations. Normal feathered chicken has the highest number of rare alleles and can be selected for increase production. All the microsatellite markers were effective in detecting rare alleles but ADL 171 was more promising in detecting rare alleles. The information on heterozygosity levels, rare alleles and inbreeding coefficient for individual/populations can be used to design breeding programmes which will maximize the genetic variation in successive generation.

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AUTH0RS
First Author[1,2]: Oni O.A, **oni_ade149@yahoo.com**
Second Author[1]: Olwofeso O, **olowofesoo@funaab.edu.ng**
Third Author[1]: De Campos J.S[1], **johndecampos@yahoo.fr**
Fourth Author[3]: Emiloju C.O[3], **opeyemi.emiloju@gmail.com**
Correspondence Author[1,2*]: Oni O.A, **oni_ade149@yahoo.com**

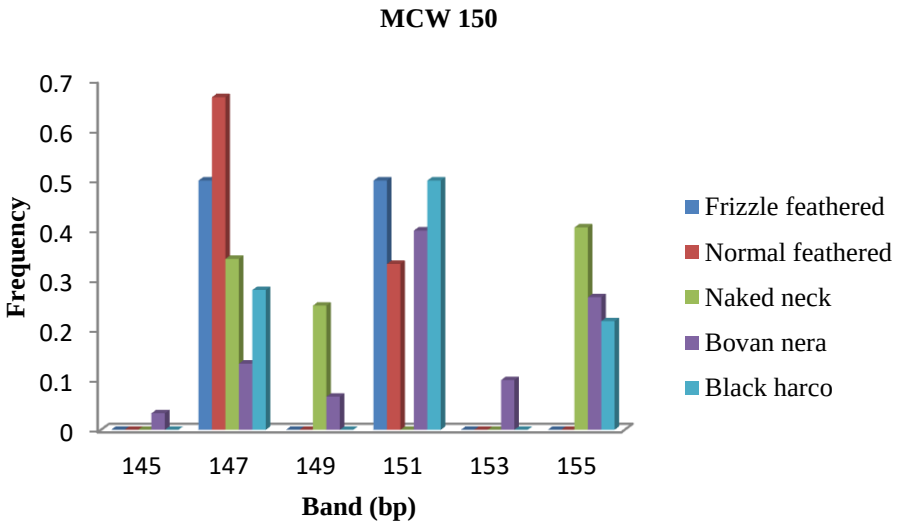

Figure 1: Allele size (bp) and allele frequencies distribution produced by MCW 150 in five chicken populations in Ogun and Ondo States, Nigeria.

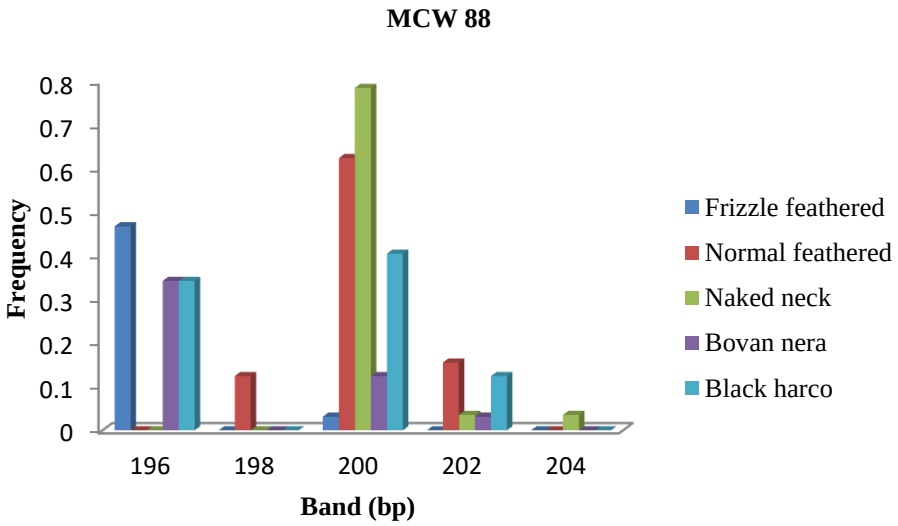

Figure 2: Allele size (bp) and allele frequencies distribution produced by MCW 88 in five chicken populations in Ogun and Ondo States, Nigeria.

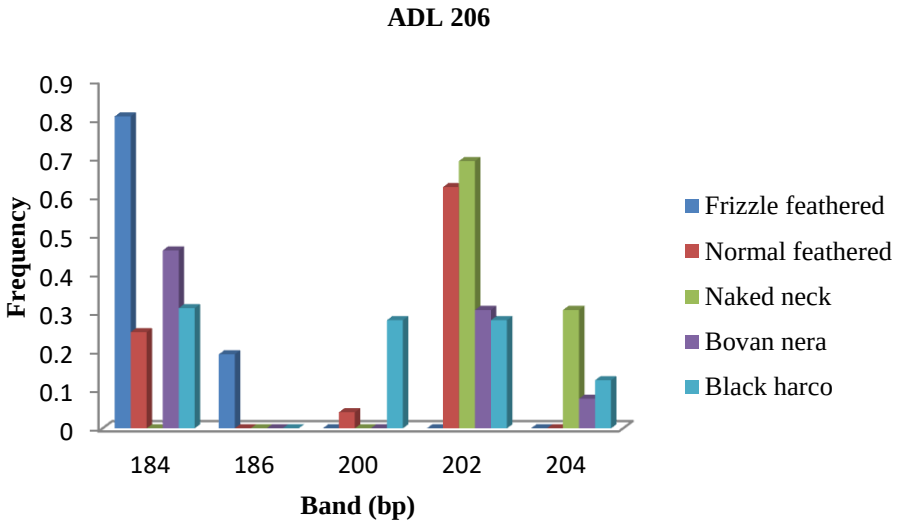

Figure 3: Allele size (bp) and allele frequencies distribution produced by ADL 206 in five chicken populations in Ogun and Ondo States, Nigeria.

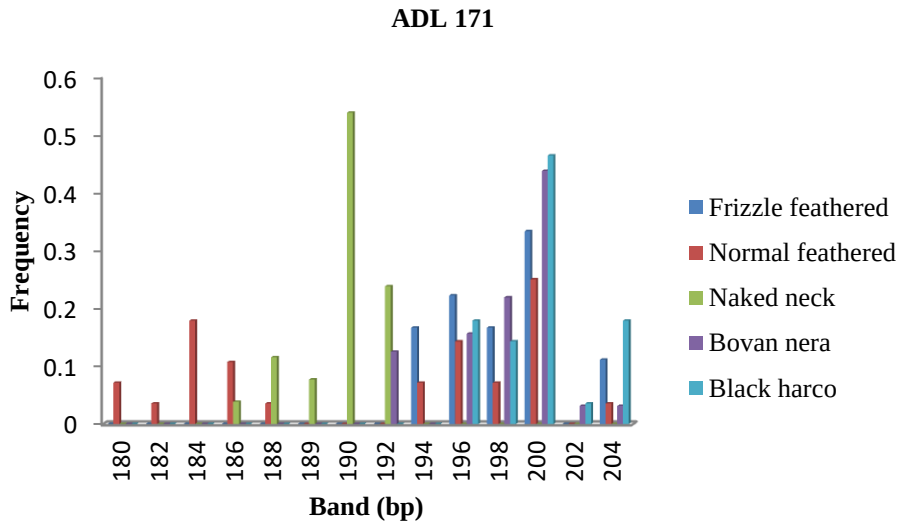

Figure 4: Allele size (bp) and allele frequencies distribution produced by ADL 171 in five Nigerian chicken populations in Ogun and Ondo States, Nigeria.

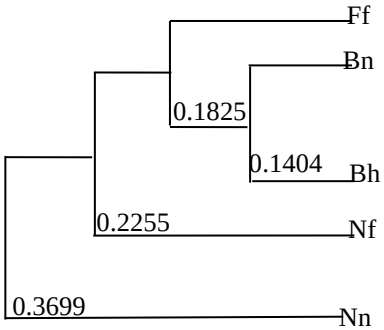

Figure 5: Dendrogram based on D_a-values of microsatellites data (using the minimum distance clustering method) and the numbers at the branch represents measure of relatedness

Table 1: Rare/Unique alleles (bold values) produced by MCW 150 in five Nigerian chicken populations in Ogun and Ondo states, Nigeria

Band/fragment size	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black harco
145	0.0000	0.0000	0.0000	**0.0333**	0.0000
147	0.5000	0.6667	0.3434	0.1333	0.2813
149	0.0000	0.0000	0.2500	0.0667	0.0000
151	0.5000	0.3333	0.0000	0.4000	0.5000
153	0.0000	0.0000	0.0000	**0.1000**	0.0000
155	0.0000	0.0000	0.4063	0.2667	0.2188

Table 2: Rare/unique alleles (bold values) produced by MCW 88 in five Nigerian chicken populations in Ogun and Ondo states, Nigeria

Band/fragment size	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black Harco
196	0.4688	0.0000	0.0000	0.3438	0.3438
198	0.0000	**0.1250**	0.0000	0.0000	0.0000
200	0.0313	0.6250	0.7857	0.1250	0.4063
202	0.0000	0.1563	0.0357	0.0313	0.1250
204	0.0000	0.0000	**0.0357**	0.0000	0.0000

Table 3: Rare/Unique alleles (bold values) produced by ADL 206 in five Nigerian chicken populations in Ogun and Ondo states, Nigeria

Band/fragment size	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black Harco
184	0.8077	0.2500	0.0000	0.4615	0.3125
186	**0.1923**	0.0000	0.0000	0.0000	0.0000
200	0.0000	0.0416	0.0000	0.0000	0.2813
202	0.0000	0.6250	0.6923	0.3077	0.2813
204	0.0000	0.0000	0.3077	0.0769	0.1250

Table 4: Rare/Unique alleles (bold values) produced by ADL 171 in five Nigerian chicken populations in Ogun and Ondo States, Nigeria

Band/fragment size	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black harco
180	0.0000	**0.0714**	0.0000	0.0000	0.0000
182	0.0000	**0.0357**	0.0000	0.0000	0.0000
184	0.0000	**0.1786**	0.0000	0.0000	0.0000
186	0.0000	0.1071	0.0385	0.0000	0.0000
188	0.0000	0.0357	0.1154	0.0000	0.0000
189	0.0000	0.0000	**0.0769**	0.0000	0.0000
190	0.0000	0.0000	**0.5385**	0.0000	0.0000
192	0.0000	0.0000	0.2380	0.1250	0.0000
194	0.1667	0.0714	0.0000	0.0000	0.0000
196	0.2222	0.1427	0.0000	0.1563	0.1786
198	0.1667	0.0714	0.0000	0.2188	0.1429
200	0.3333	0.2500	0.0000	0.4375	0.4642
202	0.0000	0.0000	0.0000	0.0313	0.0357
204	0.1111	0.0375	0.0000	0.0313	0.1786

Table 6: F Statistics value for all loci and gene flow/migrant rate

Locus	F_{ST}	F_{IT}	F_{IS}	G_{ST}	N_m
MCW 150	**0.0880**	0.4272	0.3719	**0.1075**	**2.5909**
MCW 88	**0.2284**	0.4357	0.2686	0.2295	**0.8446**
ADL 206	0.2258	**0.6994**	**0.6117**	**0.2424**	0.8591
ADL 171	0.1245	**0.2572**	**0.1514**	0.1319	0.7580
$\bar{X}$±SEM	0.1667±0.04	**0.4549±0.09**	**0.3509±0.10**	**0.1778±0.03**	1.2632±0.44

Table 7: Level of inbreeding (F_{is}) in the five Nigerian chicken populations as revealed by four microsatellite markers

Locus	Populations				
	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black harco
MCW 150	0.6107	0.7085	0.2508	0.1104	0.3122
MCW 88	0.3060	-0.0992	0.2230	0.5040	0.2853
ADL 206	0.2754	0.8511	0.2958	0.7756	0.5817
ADL 171	0.5870	0.1818	0.4118	- 0.0276	-0.3122
$\bar{X}$±SEM	**0.4448±0.09**	0.4105±0.22	0.2954±0.04	0.3406±0.18	**0.2168±0.19**

Table 8: Nei's chord genetic distances (D_a) among the five chicken populations examined in this study

Population	Frizzle feathered	Normal feathered	Naked neck	Bovan nera	Black harco
Frizzle feathered	0.0000				
Normal feathered	0.3252	0.0000			
Naked neck	0.7020	0.3699	0.0000		
Bovan near	0.1825	0.3018	0.4353	0.0000	
Black harco	0.2427	0.2255	0.4087	0.1404	0.0000

Table 9: Heterozygosity (H_S)

Locus	Populations					$\bar{X}$±SEM
	Frizzle feathered	Normal feathered	Naked neck	Bovan Nera	Black Harco	
MCW 150	0.5138	0.4575	0.6674	0.7494	0.6361	0.6048±0.05
MCW 88	0.5403	0.5686	0.3677	0.6300	0.6996	0.5612±0.06
ADL 206	**0.3185**	0.5598	0.4369	0.6862	0.7470	**0.5497±0.08**
ADL 171	0.8072	**0.8730**	0.6539	0.7298	0.7077	**0.7543±0.04**
$\bar{X}$±SEM	0.5450±0.10	0.6147±0.09	**0.5315±0.08**	**0.6989±0.03**	0.6976±0.02	